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PERSPECTIVE: TUMOUR SPREAD—THE PROBLEMS OF LATENCY

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SUMMARY

Tumour latency, or dormancy, is a well-recognized clinical phenomenon and induction or maintenance of this state would appear to offer a novel therapeutic approach to limiting the effects of neoplastic disease. Current interest has focused on the role that neovascularization plays in this process and the consequences of shifts in the balance between angiogenic and anti-angiogenic peptides. Targeting tumour vasculature by the administration or induction of such anti-angiogenic peptides is close to clinical evaluation.

KEY WORDS—**angiogenesis; angiostatin; apoptosis; dormancy; endostatin; latency; proteases**

INTRODUCTION

A frequent clinical observation in cancers such as melanoma and breast cancer is that recurrence of overt neoplasia may occur many years after the removal, or the successful therapy, of the primary tumour.[1,2] Presumably malignant cells, shed from the primary mass, were able to remain dormant but viable all this time, only to express their tumourigenic potential at a later date as a consequence of some shift in the balance between the host and tumour. The nature of the switch(es) responsible for releasing these dormant cells from growth restraint, thus allowing them to increase in population size to become a detectable recurrence, is imperfectly understood. Several rather general proposed mechanisms of tumour cell dormancy are listed in Table I and some of these are discussed in more detail below. However, the major focus of the present review will be on a single possible mechanism of dormancy, i.e. the absence, or the selective elimination, of an angiogenic response.

Relative to other areas of tumour biology, the phenomenon of tumour dormancy has been little studied, possibly as a consequence of the paucity of suitable animal model systems.[3] If this review had been written 10–15 years previously, it quite likely would have focused primarily on the concept that dormancy largely was attributable to maintenance of a cell-mediated immune response and release from such a dormant state was a consequence of abrogation of this control.[4,5] Such a focus would have reflected both the dominant status of tumour immunology in oncological circles and the prevalence and nature of the animal models then available. Whether the recent elevation of angiogenesis to centre-stage status as a pivotal mechanism for controlling dormancy represents appropriate recognition of its true importance or whether it simply represents a shift in conceptual thinking, coupled with the more recent development of suitable model systems with which to analyse only this aspect of the phenomenon,[6] remains to be determined. It is important, though, to recognize that in matters of complex pathophysiology not only do scientific 'fashions' determine the relative weight accorded to particular mechanisms, but also that tumour cells may be maintained in a dormant state via a number of different host–tumour interactions which are not necessarily mutually exclusive.

ANGIOGENESIS AND THE CONTROL OF DORMANCY

It has become a widely accepted view that 'the growth of tumours beyond 1–2 mm^3 depends upon angiogenesis'.[7–9] Since, with currently available detection techniques, this size of tumour deposit probably is undetectable, it follows, at least theoretically, that absence of an angiogenic response represents a prime mechanism for determining dormancy as understood by the functional definition. The surgical removal of certain tumours, such as breast and colon carcinomas or osteogenic sarcomas, can be associated with the explosive growth of distant metastases.[6] Originally, in terms of vascular control of this phenomenon, it was suggested that the apparent suppressive effect which the primary tumour had on the growth of metastases might be attributable to the possibility that while the primary tumour stimulated angiogenesis in its own vascular bed, it inhibited angiogenesis in the vascular bed of metastatic deposits.[10] Based on this hypothesis, a search for a circulating angiogenesis inhibitor in the serum and urine of cancer-bearing mice ended with the discovery of angiostatin, a 38 kD fragment of plasminogen[6] generated by the proteolysis of this natural protein by a variety of enzymes.[11,12] A similar strategy in a different tumour type resulted in the identification of endostatin, a 20 kD C-terminal fragment of collagen XVIII, as another angiogenesis inhibitor.[13] These results suggest

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Table I—Possible mechanisms for maintaining tumour dormancy

Avascularity	
Failure to induce angiogenic response	
Production of anti-angiogenic peptides	
Immunity	
Cell mediated	Cytolytic/cytostatic
Humoral	
Regulation of proliferation	
Hormones	—absence of growth stimulation
Cytokines/hormones	—growth suppressive effects; induction of differentiation
Growth factors	—absence of growth stimulation

that the generation of natural cleavage products, by many of the enzymes which actually are involved in facilitating an angiogenic response,[14] may be a general procedure for the regulation of neovascularization via a process which amounts practically to a feedback mechanism.[15] Because the increased generation and unopposed activity of proteolytic enzymes, an event which plays a major role in determining endothelial cell invasion, could have deleterious effects on normal host tissue, it would seem reasonable to have the cleavage products of non-anti-angiogenic molecules exert an anti-angiogenic effect via the inhibition of further degradative activity. What is not yet clear is whether these cleavage products do indeed act in a conventional feedback mode by inhibiting proteolytic function, or whether their angiogenesis-suppressing activities are unrelated to this effect. Why such an effect does not shut down the vasculature of the primary tumour remains unclear, though it may be that the local balance between angiogenic stimulators and inhibitors varies from tumour to tumour. Functionally, any tumour in which the level of anti-angiogenic factors outweighed the level of angiogenic factors would be dormant and thus undetectable.

The treatment of mice bearing any one of three murine tumours with systemic recombinant endostatin resulted not only in the failure to induce acquired drug resistance, but, unexpectedly, in the finding that after repeated cycles of this anti-angiogenic therapy, there was a prolonged period of tumour dormancy without further therapy.[16] A similar induction of prolonged tumour dormancy was observed in a human prostate carcinoma xenograft in immunosuppressed mice following the discontinuation of angiostatin administration.[17] While the precise mechanism for this activity is obscure, it relates to the control exerted by the endothelial cells over the tumour cells. Thus, in experiments where angiostatin was used to suppress the formation of metastases, it was evident that prevention of blood vessel formation resulted in the balance between cell growth and cell loss shifting in favour of cell loss as a consequence of increased apoptosis resulting from the lack of nutrients and oxygen.[18]

The balancing of a high proliferative rate by a high apoptotic rate was also evident in experiments where cDNA coding for murine angiostatin was transfected into a murine fibrosarcoma.[18] While tumour cells themselves do not usually express angiostatin, they do express many of the proteases capable of generating this cleavage product. Alternatively, the tumours may be infiltrated with normal host cells which express such proteases.[20] The introduction of cDNAs encoding angiostatin would therefore be expected to boost or supplement the base-line levels of this molecule within the tumour mass. The experiments of Cao *et al.*[19] therefore suggested that gene therapy approaches based on the delivery of anti-angiogenic genes may be feasible.[21] It is of interest to note in this respect that the delivery of a portion of the angiostatin gene by recombinant adenovirus has resulted in the *in vivo* regression of tumour.[22] Unlike many other gene therapy protocols, there are reasons to suggest that secondary tumours might represent relatively accessible potential targets for this approach. The major problem which bedevils all gene therapy approaches is that of gene delivery. Since the ultimate therapeutic intention is likely to be to switch off the angiogenic activity in metastases, released from any inhibitory effect of the primary mass, the need is for the systemic delivery of an agent which is capable of affecting or localizing to disseminated sites. This, though, mimics the natural biology of angiostatin and endostatin, originally found in the serum of tumour-bearing animals,[6] which exert their effect on metastatic locations. Thus, boosting angiostatin production by the primary tumour mass, or introducing non-transformed genetically-manipulated cells which could serve as 'protein factories', might obviate the need to deliver the therapeutic gene directly to secondary sites, which is such an intractable problem in many gene therapy approaches.

The role that the various proteolytic enzymes play in regulating angiogenesis is intricate and far from clear. As indicated above, these proteases are intimately involved both in the invasive/metastatic capacity of malignant tumours and in the invasive activity of normal endothelial cells during the angiogenic response.[14] Yet, by virtue of their generation of anti-angiogenic peptides, they appear to play a similarly vital role in anti-angiogenic activities.[20] At the time of writing, it is not apparent whether all proteolytic enzymes are comparable with regard to these disparate activities, or whether specific enzymes have dominant roles in one or other of these functions. For example, the down-regulation of the cell surface urokinase plasminogen activator receptor (uPAR), which serves to focus uPA activity at the cell surface, not only results in a

depression of invasive and metastatic activity of malignant cells, but also forces them into a protracted state of dormancy.[23] Given the anti-angiogenic activity of the cleavage products of plasminogen,[11] it might be thought that any down-regulation of uPA activity would result in a shift from the anti-angiogenic to the angiogenic balance in such tumours. Does this then mean that uPA plays no part in generating anti-angiogenic peptides? Do the findings in this one tumour type have a more general relevance? Is the induced dormancy status attributable to the changes in proteolytic activity, or is it a consequence of alterations in unrelated activities, such as cell adhesion? Questions such as these serve to highlight how little we truly know about the biochemical regulation of angiogenic activity and how much there is to determine in order to manipulate the system for therapeutic benefit.

IMMUNITY AND THE REGULATION OF DORMANCY

As stated in the Introduction, much of the early work with animal tumour models focused on the role of cell-mediated immunity in maintaining dormancy.[4] However, with the realization that many animal tumours were considerably more immunogenic than their human counterparts, enthusiasm for this explanation has waned in recent years. None the less, there are still reasons to believe that these aspects of host–tumour interaction could play an important role in the phenomenon.

The belief that dormancy is to some extent dependent on a functioning immune system is fostered by the finding that the incidence of certain tumours, specifically non-Hodgkin's (B-cell) lymphomas and virally-induced urogenital tract tumours, increases in patients who have been immunosuppressed after organ transplantation.[24] However, as Uhr *et al.*[24] cautioned, these two tumour types possess certain characteristics which render them particularly susceptible to this mode of growth regulation and their behaviour thus may prove the exception to the rule.[24] Whether the recent demonstration and cloning of specific human tumour antigens means that the immune-mediated maintenance of dormancy should be re-visited in more detail again founders on the lack of suitable animal models. For this reason, the possibility that alterations in host immune status may lead to emergence from dormancy remains an attractive, but unproven, possibility.

HOST ENVIRONMENT AND THE REGULATION OF DORMANCY

In 1959, the Fishers[25] showed that intraportal injection of Walker 256 cells into rats failed to produce obvious tumours in the liver. That this absence of growth was a consequence of dormancy was suggested by the fact that subsequent hepatic trauma led to the development of tumours within a few weeks.[25] Presumably the trauma had altered the host in some fashion such that the growth of quiescent tumour cells could no longer be inhibited and indeed now was stimulated. While a putative explanation could be that various cytokines and growth factors, necessary to support the growth of the arrested tumour cells, are missing in the normal organ but present in the damaged organ, there is scant evidence available to support such a contention.

In hormone-dependent cancers, it might be that growth restraints on tumour cells are removed either when tumour variants arise which elaborate their own growth factors, or a mutation constitutively activates the appropriate growth factor receptor, or when a change occurs in the host which results in enhanced provision of the required hormone to the tumour. Such a mechanism possibly could be the physiological basis for the oft-cited contention that a 'positive attitude' to cancer may equate with a more favourable prognosis. If the positive attitude is a reflection, or cause, of variable hormonal levels, then the regulatory growth effects which such hormones exert on tumour cell growth might well translate into variations in prognosis.[26]

However, even with such possibilities it is impossible to escape from the ubiquitous influence of the angiogenic response. It is of interest that a recent report[27] has suggested that elevated levels of certain hormones, such as occur physiologically with increasing age, might influence tumour growth not by a direct effect on the tumour cell themselves, but via the induction of tumour neovascularization.[27]

Schiffenbauer *et al.*[27] reported that elevated levels of gonadotropins [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)] promoted the growth of ovarian carcinoma via the induction of an angiogenic response. This enhanced angiogenesis was caused by the elevated secretion of vascular endothelial growth factor (VEGF) induced by the raised levels of LH and FSH.[27] It was suggested that hormonal therapy, aimed at lowering circulating levels of gonadotropins, could prolong remission in ovarian cancer by extending tumour dormancy.[27] Irrespective of whether or not such an approach might be useful clinically, these observations serve to emphasize that currently, the regulation of tumour angiogenesis appears to be the single most important means of determining tumour dormancy.

CONCLUSION

The maintenance, or induction, of tumour latency or dormancy, whether by gene therapy, hormonal manipulation or the administration of novel agents, represents an attractive therapeutic approach to different cancers. Understanding the precise basis of dormancy is not, then, simply important for the intellectual satisfaction which such knowledge brings, but also for the possibility that novel therapeutic agents will be discovered as a direct consequence of such knowledge.

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